

Appln. No. 09/529,172

Supplemental Amdt. dated June 4, 2004

Reply to Office action of November 18, 2003

Amendments to the Claims:

This listing of the claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1 (Currently Amended). A genetically stable Lemnaceae plant comprising foreign DNA that has been transformed by an *Agrobacterium*-mediated method, and progeny thereof that have inherited the transformation foreign DNA.

2 (Previously Presented). A transformed *Lemnaceae* plant according to Claim 1, of the genus *Spirodela*, *Lemna* or *Wolffia*.

3 (Original). A transformed *Lemnaceae* plant according to Claim 2, being *Spirodela punctata* of strain 8717.

4 (Previously Presented). A transformed *Lemnaceae* plant according to any one of Claims 1 to 3, that is transformed so as to be antibiotic resistant.

5 (Original). A transformed *Lemnaceae* plant according to Claim 4, being resistant to kanamycin.

6 (Previously Presented). A transformed *Lemnaceae* plant according to claim 1, that is transformed so as to be herbicide resistant.

7 (Previously Presented). A transformed *Lemnaceae* plant according to Claim 6, that is transformed so as to be

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tolerant to oxynil herbicides, to glyphosphate and EPSPS inhibitor herbicides, to glufosinate or to HPPD inhibitors.

8 (Currently Amended). A transformed *Lemnaceae* plant according to claim 1, ~~capable of expressing~~ comprising two or more foreign genes.

9-11 (Cancelled).

12 (Previously Presented). A method for the stable genetic transformation of *Lemnaceae* whole plants, plant tissue or callus, which comprises:

bringing the *Lemnaceae* whole plant, plant tissue or callus into contact with *Agrobacterium* cells containing a transforming DNA molecule; and

incubating the *Lemnaceae* whole plant, plant tissue or callus with the *Agrobacterium* cells, whereby cells in said whole plant, plant tissue or callus become stably transformed with said DNA.

13 (Currently Amended). A method according to Claim 12, wherein the *Agrobacterium* cells ~~are capable of~~ specifically targeting the plant's meristematic tissue.

14 (Previously Presented). A method according to Claim 13, wherein the *Agrobacterium* cells are *A. tumefaciens* strains BHA105, EHA101 or GVE3103.

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15 (Currently Amended). A method according to Claim 12, wherein the *Agrobacterium* cells ~~are capable of~~ targeting wounded regions in the plant.

16 (Previously Presented). A method according to Claim 15, wherein the *Agrobacterium* is *A. tumefaciens* strains LBA4404 or C58.

17 (Previously Presented). A method according to claim 12, wherein during the incubation of the *Lemnaceae* plant tissue with the *Agrobacterium* cells vacuum infiltration is applied.

18 (Original). A method according to Claim 12, wherein prior to incubation of the *Lemnaceae* plant tissue with the *Agrobacterium* cells the plant's meristematic zone is exposed by removal of the daughter fronds.

19 (Previously Presented). A method for the genetic transformation of a *Lemnaceae* plant, comprising:

cutting the plant into particles of a size such that they still contain undamaged meristematic tissue capable of developing into full plants;

incubating said particles with *Agrobacterium* cells containing transforming DNA molecules, whereby said transforming DNA is introduced into meristematic cells in said particles; and

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producing transformed plants from the transformed meristematic tissue.

20 (Cancelled)

21 (Previously Presented). A method according to Claim 19, wherein the particles have diameters, the average of which is above 150 μm .

22 (Previously Presented). A method according to Claim 21, wherein the particles have diameters, the average of which is about 150 μm to about 750 μm .

23 (Previously Presented). A method for the stable genetic transformation of a *Lemnaceae* plant, comprising microinjecting *Agrobacterium* cells containing a transforming *Agrobacterium* DNA into the meristematic zone of the plant, whereby the meristemic tissue becomes stably transformed with said DNA.

24 (Original). A method according to Claim 23, carried out *in planta*.

25 (Currently Amended). A method for the *in planta* transformation of *Lemnaceae* plants, comprising:

i. exposing the plant's meristematic zone by removal of the daughter fronds;

ii. incubating the plant with *Agrobacterium* cells ~~capable of that~~ targeting to the meristemic meristematic tissue.

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26 (Previously Presented). A method according to Claim 25, wherein the *Agrobacterium* cells are *A. tumefaciens* strains EHA105, EHA101 or GVE3103.

27 (Currently Amended). A method according to claim 12, wherein the *Agrobacterium* cells are brought into contact, prior or during the transformation method, with a booster medium ~~capable of enhancing~~ that enhances the *Agrobacterium* cell's virulence, said booster medium comprising a fresh cell suspension of dicotyledonous plants or comprising *Lemnaceae* plant extracts.

28 (Previously Presented). A method according to claim 12, wherein the transformation process takes place in a media having a pH below about 5.2.

29 (Previously Presented). A method according to Claim 27, wherein the booster medium comprises a fresh cell suspension obtained from a dicotyledonous plant.

30 (Previously Presented). A method according to claim 29, wherein the fresh cell suspension is at a concentration of 1-10% (w/v).

31 (Previously Presented). A method according to claim 27, further comprising caffeine at a concentration of 100-500 mg per liter of medium.

32 (Previously Presented). A method according to claim 29, wherein the fresh cell suspension of a

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dicotyledonous plant is obtained from the family of
Solanaceae.

33 (Previously Presented). A method according to claim 27, wherein the booster medium is a plant culture medium having a pH of about 3.5 to 4.2, and comprising 1-10% (w/v) of fresh cell suspension of *Nicotiana tabacum* and 100-500 mg per liter of caffeine.

34 (Original). A method according to Claim 27, wherein the booster medium comprises a *Lemnaceae* plant extract.

35 (Original). A method according to Claim 34, wherein the *Lemnaceae* plant extracts are *Spirodela punctata* extracts.

36 (Previously Presented). A transformed *Lemnaceae* plant obtained by the method of any one of Claims 12 to 19 or 21 to 35.

37-53 (Cancelled).

54 (Previously Presented). A method of production of a product of interest, comprising growing a transformed *Lemnaceae* according to claim 1, encoding said product, in an appropriate culture medium, under conditions enabling the production of said product of interest.

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55 (Original). The method as claimed in claim 54, wherein the product of interest is further isolated and purified.

56 (Previously Presented). A method as claimed in one of claims 54 and 55 wherein the product of interest is a chemical or a biological product.

57 (Original). A method as claimed in claim 56, wherein the product of interest is selected from the group consisting of polypeptides, proteins, carbohydrates, lipids, alkaloids, pigments or vitamins.

58 (Previously Presented). A method according to Claim 34, wherein the *Lemnaceae* is *Spirodela*.

59-64 (Cancelled)

65 (Previously Presented). A method for the stable genetic transformation of *Lemnaceae* plant tissue, comprising:

inoculating *Lemnaceae* tissue with *Agrobacterium* containing a transforming DNA molecule having a heterologous DNA of interest; and

co-cultivating the tissue with the *Agrobacterium* to produce the stably transformed *Lemnaceae* tissue.

66 (Previously Presented). A stably transformed *Lemnaceae* plant, comprising a heterologous DNA of interest integrated into the chromosome, wherein said plant is produced via an *Agrobacterium*-mediated method.

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67 (Previously Presented). A method of production of a product of interest, comprising:

culturing a stably transformed *Lemnaceae* plant that expresses at least one heterologous product, which plant has been transformed by an *Agrobacterium*-mediated method; and

isolating and purifying said at least one heterologous product.

68 (Previously Presented). The transformed *Lemnaceae* plant according to claim 2, being *Lemna gibba* Hurfeish.

69 (Previously Presented). The transformed *Lemnaceae* plant according to claim 2, being *Spirodela oligorrhiza*.

70 (Previously Presented). The method of claim 23, wherein the *Lemnaceae* plant is *Spirodela oligorrhiza*.

71 (Previously Presented). A method of production of a product of interest, comprising:

culturing a stably transformed *Lemnaceae* plant that expresses at least one heterologous product, which plant has been transformed by an *Agrobacterium*-mediated method.